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Chloride uptake into cultured airway epithelial cells from cystic fibrosis patients and normal individuals

(genetic defect/permeability/nasal polyp)

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ABSTRACT **The chloride permeability of airway and sweat ductal epithelium of cystic fibrosis (CF) patients is decreased. This abnormality could represent an intrinsic characteristic of the epithelial cell or the response to a tonic extrinsic stimulus, *in vivo*. We cultured airway epithelial cells derived from CF and non-CF individuals under identical conditions that were free from donor-specific factors. Differences in the characteristics of cells that multiplied under these circumstances are unlikely to reflect the effects of extrinsic modulation present in the host. After 8–12 days in culture, the cells of CF and non-CF patients were similar in morphology and intracellular electrolyte content, but the CF cultures took up chloride at a reduced rate. The difference could not be attributed to a higher intracellular potential in CF cells or to the presence of a stilbene anion-sensitive chloride–chloride exchange in non-CF cells. We conclude that epithelial cells from CF patients grown in the absence of extracellular factors of the host express reduced cellular chloride permeability, a defect similar to that found *in vivo* and in freshly excised nasal epithelium.**

It has been difficult to accommodate the complex pattern of abnormal exocrine secretions in cystic fibrosis (CF) within a "unified" hypothesis of a single lesion in epithelial cell function (1). Our understanding of the disease has been impeded by the absence of a suitable animal model and by an insufficient supply of specimens from patients for extensive *in vitro* research (2). In addition, when abnormalities associated with CF are demonstrated *in vivo* or in freshly excised tissue, it is not obvious whether the abnormality is the direct consequence of intrinsic dysfunction or a response to factors evoked by chronic disease (3).

The recent discoveries that both airway (4, 5) and sweat ductal (6, 7) epithelia of CF patients are characterized by an abnormally small Cl^- permeability raise the expectation that the pattern of exocrine dysfunction in CF can be understood. New techniques for growing human airway epithelial cells in culture (8) could speed realization of this expectation by providing more suitable material for study. The utility of such an approach depends, however, on the demonstration that the disease is expressed in cultured cells. Phenotypic expression in cultured cells can be regulated by the concentration of modulatory substances to which the cells are exposed in culture (9, 10). It follows that any differences in the functions of cells from CF and normal subjects cultured under identical artificial conditions are more likely to represent intrinsic expressions of the disease rather than secondary responses to extracellular factors produced by the host. Accordingly, we grew primary cultures of airway epithelial cells from CF and atopic (disease control) polyps and normal nasal epithelium and measured their early rate of $^{36}Cl^-$ uptake. Ion uptake into cultured epithelial cells is influenced by the properties of the upward-facing cell membrane, which has been found in other studies to contain functions of the luminal cell membranes of the intact epithelium (9, 11). We hypothesized that if reduced Cl^- permeability is an expression of a genetic defect in CF, then CF cells should take up Cl^- at a reduced rate during the period of cell multiplication in culture.

MATERIALS AND METHODS

Tissues and Subjects. All tissues were obtained from nasal reconstructions or nasal polypectomies through an arrangement approved by the Human Rights Committee of the School of Medicine of the University of North Carolina at Chapel Hill. Disease subjects were patients with CF, diagnosed by standard clinical and sweat electrolyte criteria, who required surgery for nasal polyps. Normal nasal tissue was inferior turbinate that was removed during reconstructive procedures. Some CF and atopic patients had received medications chronically (e.g., antibiotics and steroids), but these were usually discontinued 7–14 days before surgery.

Cell Isolation and Culture. The culture conditions were described in detail by Wu *et al.* (8). Freshly excised polyp or nasal turbinate mucosa was rinsed and incubated in sterile Joklik's modified Eagle's medium with 0.1% protease (Sigma, type 14), penicillin (60 units/ml), streptomycin (60 μg/ml), and gentamicin (50 μg/ml). The tissue and medium were held at 4°C for 36–48 hr with periodic gentle agitation. For tissues obtained from distant centers, this period included transit via commercial carrier. The suspension of free epithelial cells that were detached by protease digestion was passed through nylon mesh to remove clumps and debris. The cells (ciliated, mucosal, and basal) were centrifuged (500 $\times$ *g*; 5 min) and resuspended in protease-free medium. The centrifugation and resuspension steps were repeated twice. Washed cells were seeded on 35-mm plastic tissue culture dishes (Falcon) at a density of 2×10^5 cells per dish in 2 ml of serum-free, growth factor- and hormone-supplemented Ham's nutrient mixture F-12 (8). The cells were incubated at 37°C in a humidified 5% CO_2 atmosphere. Cells not attached to the culture dish (attachment efficiency was 20–30%) were removed after 24 hr by aspiration, and the medium was replaced with fresh supplemented F-12. The cells were allowed to grow for another 5–9 days. The medium was changed every other day. Two to four days before the cells were used, when they were $\approx$70% confluent, the growth medium was changed to a 1:1 mixture of supplemented F-12 medium and Dulbecco's modified Eagle's medium/1% fetal bovine serum that had been conditioned by exposure to rat

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Abbreviation: CF, cystic fibrosis.

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3T3 fibroblasts (8). Conditioned medium appeared to further increase cell density and confluence.

Uptake Studies. Cells on plastic dishes were washed three times and preincubated at 37°C with a Na-Ringer's solution of 115 mM NaCl, 25 mM $NaHCO_3$, 2.3 mM K_2HPO_4, 0.4 mM KH_2PO_4, 1.25 mM $CaCl_2$, 1.25 mM $MgCl_2$, 10 mM Hepes (titrated to pH 7.4 with NaOH), and 5 mM glucose. When the solution [Cl^-] was changed by sulfate replacement, sucrose was added to maintain the osmolarity at 270–285 mosM. Cells were preincubated for 90–180 min in a solution with a chemical composition identical to that used for the subsequent uptake experiment. Preincubation periods were selected so that the total of preincubation and incubation periods for each cell culture was the same. Preincubation was ended by aspiration of the preincubation medium, and uptake was assessed by addition of 1.2 ml of a solution containing $^{36}Cl^-$ at 1 μCi/ml (1 Ci = 37 GBq) and/or [^{14}C]mannitol at 2 μCi/ml. The cells were exposed to uptake solution at 37°C for 2 sec–120 min. The incubation periods were timed to within 10% of the nominal value by a countdown timer with both visual and audio cues. For example, 36 "2-sec" intervals were found by a second observer with a stopwatch to average 2.24 ± 0.2 sec. At the end of the uptake period, the incubation solution was aspirated from the dish and the cells were washed four times with 5 ml of Cl^--free solution at 4°C. These wash conditions were chosen to minimize the possible contributions of diffusion and Cl^-–Cl^- exchange to the loss of isotope from the cultured cells during the wash procedure. Similar strategies for washing have been used in other studies of rapid ion uptakes (11, 12). The entire wash procedure was completed in <8 sec. The end of the uptake period was taken as the beginning of the first wash.

Each dish was extracted overnight in the cold with 2.0 ml of 0.1% $NaDodSO_4$ in water. The dish contents were dispersed by vigorous pipetting. Duplicate 50-μl samples of the extract were analyzed for protein by the Bradford technique (13); A_{595} was measured, results are expressed as mg of protein per dish. For analysis of $^{36}Cl^-$ or [^{14}C]mannitol (both from New England Nuclear) uptake, 1.6 ml of the extract was mixed with 18 ml of Aquasol II (New England Nuclear) and radioactivity was measured in a Packard Model 300 liquid scintillation counter with external-standard quench-correction. Channel limits were selected to avoid "spill" of ^{14}C activity into the $^{36}Cl^-$-counting channel. The spill of $^{36}Cl^-$ activity into the ^{14}C channel was calculated from the ratio of ^{14}C channel to $^{36}Cl^-$ channel counts from a standard with only $^{36}Cl^-$. Three 10-μl samples of the uptake medium were extracted and assayed for radioactivity in the same way. The Cl^- taken up was calculated from radioactivity in the cells and the specific activity of the uptake solution. Results are expressed as nmol of Cl^- taken up per mg of protein. The volume of distribution of [^{14}C]mannitol was calculated from the ^{14}C activity of the incubation solution and the ^{14}C and protein contents of each dish. These results are expressed as μl per mg of protein. Two to four determinations at each time were averaged.

***methyl*-^{14}C]Methyltriphenylphosphonium ([^{14}C]$MePh_3P^+$) Distribution.** Intracellular potential difference was estimated from the cellular accumulation of [^{14}C]$MePh_3P^+$ (obtained as the iodide salt from New England Nuclear), a lipophilic cation that has been used to estimate the intracellular potential difference of a number of cell types (14), including canine tracheal epithelial cells (15). Cells on dishes were exposed to Na-Ringer's solution with [^{14}C]$MePh_3PI$ (0.1 μCi/ml; 10 mCi/mmol) and [^{3}H]inulin (1 μCi/ml; 500 Ci/mmol) (New England Nuclear). After 5, 10, 30, 45, and 60 min, the cells were washed twice with Ringer's solution and extracted for liquid scintillation counting as described above. After 30 min, some cultures were exposed to 0.1 mM carbonylcyanide *m*-chlorophenylhydrazone (CCCP, Sigma) and incubated for an additional 30 min. Replicate 50-μl samples of the incubation medium were processed similarly. The ^{14}C radioactivity contained in the [^{3}H]inulin space was <5% of the total ^{14}C radioactivity. The intracellular potential difference was estimated from the ratio of extracellular and intracellular ^{14}C. The results from two or three cultures per incubation interval were averaged.

Electrolyte Analysis. Intracellular concentrations of Na^+, K^+, and Cl^- were estimated from the electrolyte content of the pool of cells that were detached from 8–12 dishes of cells per patient by trypsin (0.1%, 5 min, 37°C). Trypsin was removed by centrifuging and resuspending the cells in trypsin-free Kreb's bicarbonate Ringer's solution. The cells were then incubated with an extracellular marker, [^{3}H]inulin, for 30 min at 37°C in an atmosphere of 95% O_2/5% CO_2. The cell suspension was divided among several plastic centrifuge tubes and spun (Fisher Model 235A microfuge, 12,000 × *g*) for 90 sec. Duplicate 50-μl samples of each supernatant were taken, and the remaining liquid was aspirated. The tip of each tube, which contained a cell pellet, was excised with a razor blade and weighed. The cell pellet and tube tip were dried overnight at 95°C, reweighed, and then shaken with 2 ml of ultrapure 0.1 M HNO_3 (Baker) in acid-washed glass vials for 48 hr. The plastic tip was recovered, washed, dried, and weighed. Samples of the dried supernatant liquid were extracted the same way. The acid extract was analyzed for sodium and potassium content by flame photometry, for chloride content by amperometric titration, for [^{3}H]inulin content by liquid scintillation counting and for protein as described above. The inulin space and its associated ion concentration were subtracted from the total water and ion concentrations of the cell pellet, respectively. The water that remained (intracellular water) is expressed as μl per mg of protein. Intracellular ion concentrations are expressed as mmol per kg of intracellular water or per mg of protein.

The results are expressed in the text as the mean ± SEM. Differences between means were analyzed for statistical significance by unpaired or paired *t*-tests as appropriate. The slopes, intercepts, and correlation coefficients of initial uptake rates were obtained from least-square analyses.

RESULTS

The cells grown in tissue culture by this technique have been shown to be epithelial in origin by two criteria: (*i*) they stain positively for keratin in immunofluorescence studies (8) and (*ii*) removal from primary culture in log phase and inoculation into denuded rabbit tracheas, which are then implanted into athymic mice, results in repopulation of the rabbit tracheal basement membrane with a fully differentiated and ciliated epithelium (16). The nasal epithelial cells grown in tissue culture on plastic dishes, however, differ in morphology from the primary isolates. They are flattened and lack some important morphologic features of respiratory epithelia, including cilia and mucus granules (Fig. 1 and ref. 8). Cells from CF subjects had the same general general appearance as those of non-CF subjects (Fig. 1).

The growth characteristics of cells derived from CF and normal or atopic subjects were also similar. For example, CF and non-CF cells had a doubling time of ≈24 hr in serum-free medium. Non-CF (14 patients) and CF (11 patients) cultures inoculated at 10^5 cells per dish reached confluence in 11.7 ± 0.4 and 11.2 ± 1.6 days, respectively. At the time of uptake studies, the protein content per dish, an index of cell number, was similar for the two populations (0.372 ± 0.024 mg for non-CF and 0.432 ± 0.052 mg for CF).

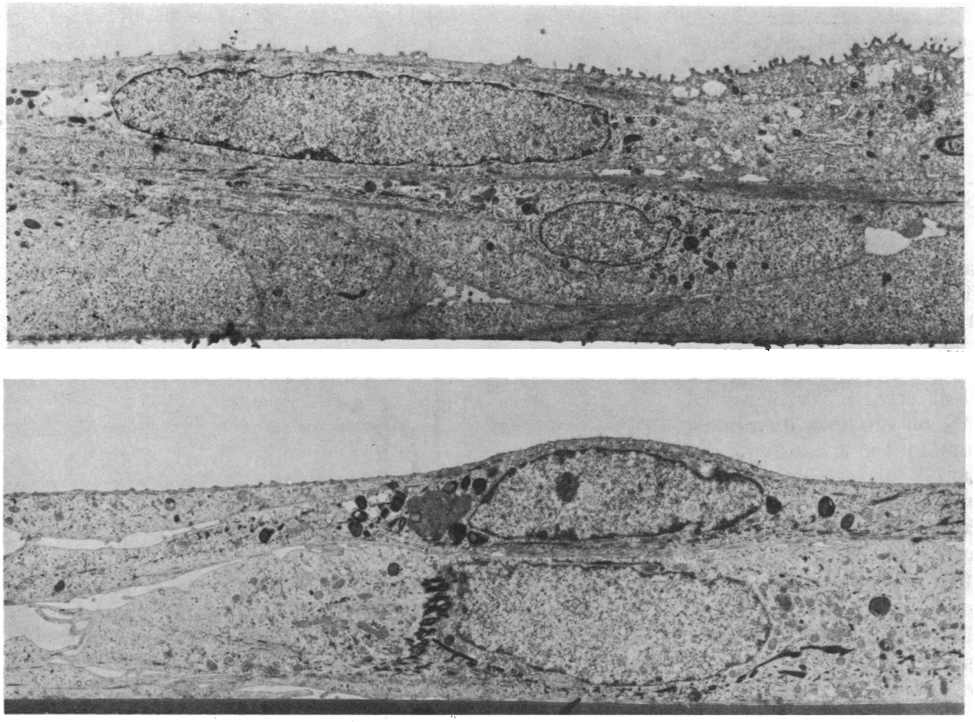

FIG. 1. Thin-section electron micrographs of cultured human nasal epithelial cells from a CF (*A*) and a non-CF (*B*) patient. Note that both preparations are multilayered and nonciliated. The apparent greater density of microvilli in the CF cells has not been seen in every section and a detailed morphological analysis has not been performed. (Uranyl acetate/lead citrate stain; ×2500.)

The time-course of Cl^- uptake by cultured CF and non-CF cells* is shown in Fig. 2. Uptake in both groups approached steady-state by 30 min. The volume of distribution of [^{14}C]mannitol also reached steady-state by 30 min (non-CF, 6.58 ± 1.95 μl/mg of protein; CF, = 5.39 ± 1.14 μl/mg of protein; n = 6 for each group). In preliminary experiments, [^{14}C]mannitol and [^{3}H]inulin were shown to distribute into the same space over this interval. The steady-state Cl^- content of cells, when corrected for the tracer content of the steady-state [^{14}C]mannitol space, predicts an intracellular Cl^- concentration of 33 ± 3 and 30 ± 7 mmol/kg of intracellular H_2O for non-CF and CF cells, respectively. These estimates are ≈50% of the intracellular Cl^- concentration obtained by chemical analyses of detached cells (Table 1). The results of both analyses indicate that the intracellular Cl^- concentrations of CF and non-CF derived cells were not different.

Whereas there was no difference between steady-state Cl^- concentration of CF and non-CF cells (Fig. 2 and Table 1) the early rate of $^{36}Cl^-$ uptake by the CF cells was only 65% that of non-CF cells (Fig. 3). A line was fitted to the uptakes at 2, 10, and 20 sec by linear-regression analysis. Although uptake by both groups of cells tended to slow with time, the data are described by two highly correlated linear relationships. The slopes or rates of uptake are statistically different ($P < 0.025$) but the intercepts on the y axis are not ($P < 0.4$). In contrast, the [^{14}C]mannitol volume of distribution at 2 sec (0.75 ± 0.28 μl/mg for non-CF and 0.87 ± 0.13 μl/mg for CF) and 20 sec (1.91 ± 0.37 μl/mg for non-CF and 2.12 ± 0.28 μl/mg for CF) was nearly identical for the two groups.

The rate of uptake in both experimental groups increased linearly with Cl^- concentration (Fig. 4). At each extracellular Cl^- concentration tested, the rate of Cl^- uptake into non-CF cells exceeded that into CF cells. The slope of the relationship between uptake and concentration yielded permeation coefficients of 0.165 ± 0.002 $\mu l \cdot mg^{-1} \cdot sec^{-1}$ for non-CF and 0.093 ± 0.004 $\mu l \cdot mg^{-1} \cdot sec^{-1}$ for CF cells ($P < 0.001$). The linear increase in uptake with concentration is consistent with a nonsaturable process.

The smaller Cl^- uptake into CF cells could reflect either missing paths for Cl^- entry or a less favorable electrochemical driving force for entry. Cl^- uptake by some cell types includes a component of Cl^-–Cl^- exchange. Often this exchange is inhibited by stilbene sulfonic acids such as 4,4′-diisothiocyanatostilbene-2,2′-sulfonic acid (DIDS) or 4-acetamido-4′ isothiocyanotostilbene-2,2′-sulfonic acid (SITS) (12, 17). We attempted to determine whether the greater uptake into non-CF cells resulted from the existence of a Cl^- exchange mechanism that was missing in CF cells. Cl^- uptake into cells from three non-CF patients was measured with or without 30 min of pretreatment with 0.1 mM SITS. This protocol of SITS treatment abolishes Cl^-–Cl^- exchange in cultured glial cells (12) and Ehrlich ascites cells

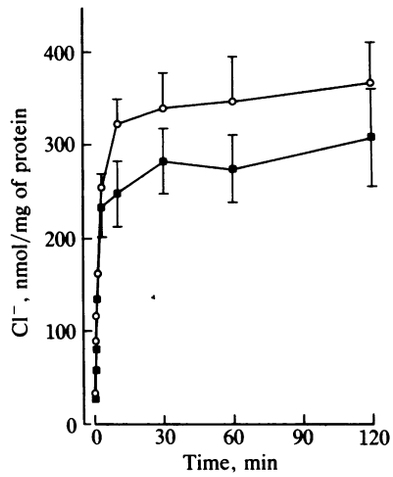

FIG. 2. Uptake of Cl^- into cells cultured from non-CF (○) and CF (■) nasal epithelia. Exposure was begun at 0 time and terminated after 2, 10, or 20 sec or 1, 3, 10, 30, 60, or 120 min. The uptake at each time in any experiment was the average of 3 or 4 replicates. Data shown are the mean ± SEM of 6 separate experiments.

*There was no difference between results for turbinate (normal) and atopic polyp (disease control)-derived cells. Consequently, uptakes by cells from these sources were combined.

Table 1. Electrolytes in cultured cells

Epithelial source	Concentration, mmol/liter of cellular H_2O		
	Na^+	K^+	Cl^-
Nasal turbinate	47.3 ± 6.7	115.9 ± 4.5	62.1 ± 5.3
Atopic polyp	51.0 ± 2.1	99.8 ± 5.6	63.3 ± 3.6
CF polyp	44.5 ± 8.7	117.0 ± 8.1	56.9 ± 5.6

Values are given as mean ± SEM (n = 4 for non-CF; n = 8 for CF).

(17) but did not affect Cl^- uptake by non-CF cultured nasal epithelial cells. Uptake in the SITS-pretreated cells was 1.23 ± 0.13 times that of the cells that were not treated ($P > 0.3$).

If cellular Cl^- uptake occurs by electrodiffusion, slower entry into CF cells could be caused by a more negative (intracellular) electrical potential difference. We measured the distribution of $[^{14}C]MePh_3P^+$, an ion that distributes across cellular and organelle membranes in accord with the potential difference (14), in non-CF and CF cells. The potential difference estimated from the steady-state distribution of $[^{14}C]MePh_3P^+$ (60 min) was 65 ± 6 mV for CF and 79 ± 6 mV for non-CF cells. In the presence of the protonophore carbonylcyanide *m*-chlorophenylhydrazone, which collapses the mitochondrial potential difference, the estimated potential difference was 51 ± 7 mV in CF and 62 ± 8 mV in non-CF cells. These potential differences are similar to those measured by the same technique in freshly disaggregated canine tracheal epithelial cells (15).

DISCUSSION

Airway epithelial cells isolated from the nasal mucosa of non-CF and CF patients grew in culture to populate plastic culture dishes with flattened, nonciliated cells. These cells were nonetheless of airway epithelial origin, because they have been shown to repopulate, with a ciliated epithelium, the basement membrane of tracheas that are implanted in athymic mice (16). Both CF and non-CF cells had doubling times of ≈24 hr and reached 70–80% confluence in 8–10 days. After exposure to conditioned medium (with 1% fetal calf serum) for 2–4 days, the CF and non-CF cultures contained nearly the same amount of protein per dish. CF and non-CF cells also had the same general morphologic appearance (Fig. 1) and ionic composition (Table 1). Although the intracellular Cl^- concentrations of all cells that were estimated from the $^{36}Cl^-$ associated with the cellular compartment at 60 min were lower than the chemical estimates, Widdicombe *et al.* (18) found a similar discrepancy in estimates of intracellular Cl^- concentration of freshly isolated dog tracheal cells by $^{36}Cl^-$ equilibration and chemical analyses. This difference between isotopic and chemical analyses of Cl^- activity may be methodological. Complete isotopic equilibration may involve a slowly exchanging compartment. It is also possible that exposure of the cells to trypsin in the chemical analysis protocol may damage the cell membrane and raise the concentrations of intracellular Cl^- and Na^+.

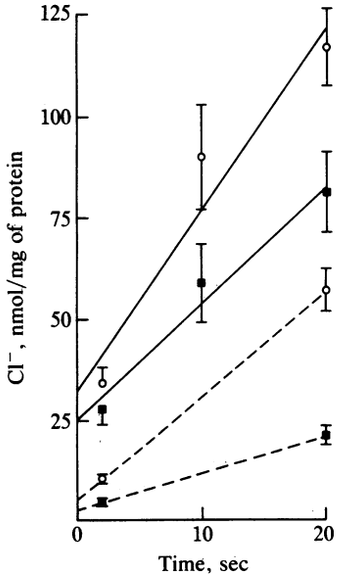

FIG. 3. Early Cl^- uptake into cells cultured from non-CF (○) and CF (■) nasal epithelia. Uptake was begun at 0 sec and terminated after 2, 10, or 20 sec. Points represent the mean ± SEM of 14 experiments with non-CF and 11 experiments with CF cells. Each point in each experiment was the average of 3 or 4 replicates. For non-CF cells, regression analysis yielded a slope of 4.67 ± 0.43 nmol of Cl^-/mg of protein per sec (r = 0.97) with a y intercept of 31.77 ± 5.68 nmol/mg. For CF cells, the slope was 2.93 ± 0.44 nmol of Cl^-/mg of protein per sec (r = 0.99) with y intercept of 24.96 ± 4.67 nmol/mg. The points on the dashed lines represent the uptake of Cl^- in excess of that calculated to be contained in the $[^{14}C]$mannitol space measured at 2 and 20 sec.

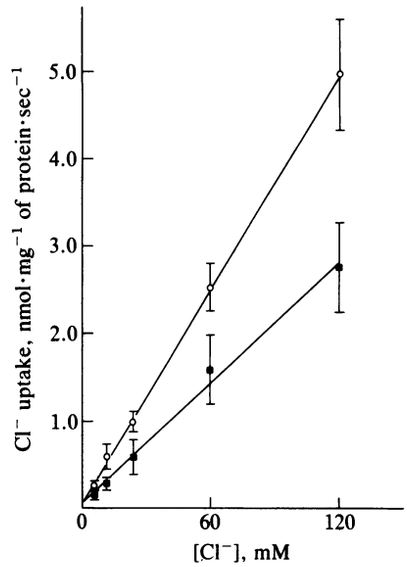

FIG. 4. The effect of bath Cl^- concentration on Cl^- uptake by non-CF (○) and CF (■) cultured airway epithelial cells. Uptakes were determined at each Cl^- concentration by the approach described for Fig. 3. Points represent the mean ± SEM of the early uptake slope from 7 separate experiments.

The early rate of $^{36}Cl^-$ uptake by CF cultures was about two-thirds of that by non-CF cultures (Fig. 3). We assumed that the early rate of $^{36}Cl^-$ uptake was an index of the permeability of the upward-facing cell membrane and permeation of tracer into extracellular compartments associated with the cell matrix, as well as a small quantity of superficial tracer that was not removed by the wash procedure. Although the intracellular compartment is of interest, it is difficult to partition the uptake of solute. We estimated the magnitude of uptake into the extracellular compartment from the $[^{14}C]$mannitol space at selected times of incubation (2 and 20 sec, 30 and 60 min). At no point was the difference between the volumes of distribution of $[^{14}C]$mannitol in the two groups statistically significant. At 2 and 20 sec, the $[^{14}C]$mannitol activity that remained after the wash was nearly identical in CF and non-CF cells. Other investigators (11, 12) have attempted to correct total uptakes for the fraction of solute that adheres to the dish by subtracting the uptake at the earliest time (a "zero time uptake") or the y intercept of the uptake vs. time plot. These estimates cannot partition the remaining $^{36}Cl^-$ uptake between intracellular and extracel-

lular compartments. Since the rates of mannitol permeation into the cell matrices and the steady-state mannitol spaces were similar in CF and non-CF cells, the difference in early $^{36}Cl^-$ uptake is likely to represent a difference in uptake into a cellular compartment and, therefore, a difference in the rate of Cl^- permeation through the upward-facing cell membrane.

Studies of the progenitor tissues *in vivo* (4) and *in vitro* (5) lead to a comparable interpretation. Knowles *et al.* (4) found a smaller Cl^- diffusion potential *in vivo* across the nasal mucosa of CF patients which was compatible with a lower relative chloride permeability of the lumen-facing membrane. The transepithelial Cl^- permeability of excised CF nasal polyp was later found to be less than that of atopic polyp or normal turbinate (5). On the basis of the ratio of Cl^- permeability coefficient to that of the extracellular marker [^{14}C]mannitol, the site of the reduced Cl^- permeability was inferred to be in the cellular path. Recently, we used microelectrodes to determine that the Cl^- conductance of the apical cell membrane of CF nasal polyps was less than that of normal turbinate or atopic polyp epithelium (unpublished data).

The ratio of early rates of Cl^- uptake into non-CF and CF cultured epithelial cells (Fig. 2), can be compared to 2.2, the ratio of Cl^- permeability coefficients for excised non-CF and CF nasal epithelium that was measured *in vitro* (5). The qualitative concordance between the patterns of Cl^- flow across freshly excised epithelia and into cultured cells suggests that the defect in apical cell membrane Cl^- permeation is maintained in culture.

Cl^- permeation of the apical cell membrane of CF airway epithelial cells could be diminished because there are fewer paths or because there is a less favorable electrochemical gradient for Cl^- entry into the cell, or both. For example, the lower uptake into CF cells could be attributed to reduced Cl^-–Cl^- exchange. This explanation is unlikely, however, for several reasons. First, Cl^- gradients across the plasma membrane of CF and non-CF cells (Table 1) were similar. Second, 10^{-4} M SITS did not affect Cl^- uptake. Third, uptake did not approach a maximum as the bath Cl^- concentration was raised (Fig. 4), a characteristic of Cl^-–Cl^- exchange processes in many cells (12, 17).

If the flow into cultured cells occurs mostly through conductive paths, then the reduced uptake in CF cells could reflect a smaller conductance (size or number of paths) or a smaller driving force. The integrated Nernst–Planck relationship between unidirectional flux and driving forces can be used to estimate the difference in transmembrane potential difference that would be required to account for the lower uptake in CF cells. If the transmembrane potential difference of non-CF cells is taken as −60 mV, then the potential difference of CF cells would have to be about −80 mV to reduce the uptake of Cl^- to the observed rate. Since all of the Cl^- uptake is not into the cell, the actual disparity in potential differences would have to be even greater to account for the cellular component of Cl^- uptake. The [^{14}C]$MePh_3P^+$ distribution in CF and non-CF cells suggests a smaller rather than larger transmembrane potential differences for CF cells, a finding that is consistent with microelectrode analyses of the intact tissue (unpublished observation). Accordingly, the simplest interpretation of our data is that CF cells have a smaller apical Cl^- conductance.

We conclude that CF epithelial cells grown in tissue culture retain a defect in cell Cl^- permeability that resembles that found in intact tissues. Because only 12% of the population of cultured cells at the time of study is comprised of the original cells that were seeded (i.e., 2×10^5 cells seeded at 30% efficiency yielded 5×10^5 cells), the Cl^- impermeability appears to be related to the expression of information contained in the genome of the epithelial cell and does not require the presence of circulating humoral factors from the host. This preparation will increase the amount of CF material available for study and can be useful for further biochemical and genetic studies of the disease in the absence of the variables that may confound the results obtained from freshly excised tissues.

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1. Davis, P. B. & Di Sant'Agnese, P. A. (1980) *Pediatr. Res.* **14,** 83–87.
2. Neutra, M. R. (1980) *Birth Defects Orig. Artic. Ser.* **16,** 261–273.
3. Wood, R. E., Boat, T. F. & Doershuk, C. F. (1976) *Am. Rev. Respir. Dis.* **113,** 833–878.
4. Knowles, M. R., Gatzy, J. T. & Boucher, R. C. (1983) *J. Clin. Invest.* **71,** 1410–1417.
5. Knowles, M. R., Stutts, M. J., Spock, A., Fischer, N., Gatzy, J. T. & Boucher, R. C. (1983) *Science* **221,** 1067–1070.
6. Quinton, P. M. & Bijman, J. (1983) *N. Engl. J. Med.* **308,** 1185–1189.
7. Quinton, P. M. (1983) *Nature (London)* **301,** 421–422.
8. Wu, R., Yankaskas, J. R., Cheng, E., Knowles, M. R. & Boucher, R. C. (1985) *Am. Rev. Respir. Dis.*, in press.
9. Sariban-Sohraby, S., Burg, M. B. & Turner, R. J. (1983) *Am. J. Physiol.* **245,** C167–C171.
10. Schimke, R. T. (1974) in *Biochemistry of Cell Differentiation*, ed. Paul, J. (Butterworth, London), pp. 183–221.
11. Rindler, M. J., Taub, M. & Saier, M. H. (1979) *J. Biol. Chem.* **254,** 11431–11439.
12. Kimelberg, H. K. (1981) *Biochim. Biophys. Acta* **646,** 179–184.
13. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
14. Ritchie, R. J. (1982) *J. Membr. Biol.* **69,** 57–63.
15. Cotton, C. U. & Gatzy, J. T. (1983) *Pharmacologist* **25,** 251 (abstr.).
16. Yankaskas, J. R., Knowles, M. R., Gatzy, J. T. & Boucher, R. C. (1985) *Lancet* **i,** 954–946.
17. Aull, F., Nachbar, M. S. & Oppenheim, J. D. (1977) *Biochim. Biophys. Acta* **471,** 341–347.
18. Widdicombe, J. H., Basbaum, C. B. & Highland, E. (1981) *Am. J. Physiol.* **241,** C184–C192.